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Determination of Isoflavones in Soybean Flours, Protein Concentrates, and Isolates

Arthur C. Eldridge

The individual and total isoflavone content in commercial soybean protein products has been determined by high-performance liquid chromatography. Dehulled, defatted soybean flours contain the following mean isoflavone content (mg/100 g): daidzin, 61.7; glycitein 7-β-glucoside, 12.9; genistin, 119.8; daidzein, 32.8; genistein, 26.7. The same isoflavones were found in soybean protein concentrates and soybean protein isolates but in decreased amounts.

Soybeans contain isoflavones (Naim et al., 1974) that have several known activities, including estrogenic (Drane et al., 1980; Kitts et al., 1980), fungitoxic (Wyman and VanEtten, 1978), and antioxidant (Pratt and Birac, 1979) properties. Because of the ever increasing use of soybean protein products in foods and feeds, it is necessary to know the concentration of these biologically active compounds in various commercial products. Only one report in the literature (Naim et al., 1974) gives any quantitative data on the concentration of isoflavones in soybeans. Therefore, this study has been conducted to determine the amount of these compounds in soybean flours, protein concentrates, and isolates.

MATERIALS AND METHODS

Samples. A dehulled, defatted soybean flour was prepared in the laboratory (Eldridge et al., 1971) from Amsoy soybeans that were grown in 1978. In addition, one sample of commercial soybean meal and eight texturized soybean flours were obtained from various manufacturers. Five commercial samples of soybean protein concentrates (products containing a minimum of 70% protein) were obtained from four manufacturers. Three processors each use a different procedure for the preparation of their concentrates (Circle and Smith, 1972). Five soybean protein isolates (products containing a minimum of 90% protein) were procured from one manufacturer.

Trade names and sources of samples are given in Table I. All samples were ground to pass a 60–80-mesh screen.

Preparation of Extracts. Ground defatted soybean flour was extracted with several solvents to determine the most suitable solvent for dissolving the soybean isoflavones. Solvents investigated were 50%, 80%, and absolute ethanol, 50%, 80%, and absolute methanol, ethyl acetate, and acetonitrile. Refluxing with 80% methanol gave the most reproducible results and maximum extraction.

Table I. Identity of Samples Used in the Study

samples	trade name or description	source[a]
flours		
A	hexane, defatted Amsoy variety, 1978 crop	1
B	Nutrisoy 7B	2
C	unflavored TVP	2
D	Textratein	3
E	Centex 300	4
F	Centex 300 SL	4
G	Centex 400	4
H	Centex 400 SL	4
I	Mira Tex	5
J	Promote III, SL	6
concentrates		
K	Response	4
L	Food protein concentrate	7
M	Pro Con 2000	5
N	Promosoy 100	4
O	GL-301	6
isolates		
P	Edi Pro N	8
Q	Edi Pro A	8
R	Supro 610	8
S	Supro 620	8
T	Supro 710	8

[a] 1, Northern Regional Research Center; 2, Archer-Daniels-Midland Co.; 3, Cargill, Inc.; 4, Central Soya Co.; 5, A. E. Staley Manufacturing Co.; 6, Griffith Laboratories, Inc.; 7, Swift and Co.; 8, Ralston Purina Co.

In the analysis of soybean products, *n*-butyrophenone, which served as an internal standard, was dissolved in 80% aqueous methanol, and an accurate volume was added to the sample. A 1-g sample with 25 mL of 80% aqueous methanol containing the internal standard was heated (boiling) on a steam bath for 4 h, cooled, and filtered through a Type AP prefilter followed by a Type HA, 0.45-μM filter (Millipore Corp., Bedford, MA).

Chromatography. The previously published chromatographic procedure (Eldridge, 1982) was followed, using a linear methanol gradient from 25 to 50% in 20 min followed by an isocratic hold period of at least 30 min. Response factors for the individual isolated glucosides and

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Table II. Effect of Time on Extraction of Isoflavones from Defatted Soybean Meal (Sample A)

		isoflavone content, mg/100 g, as is					
		80% CH_3OH, reflux, 15:1[b]					45:1,[b]
isoflavone	Naim[a]	1 h	2 h	3 h	4 h	5 h	5 h
daidzin	67	44	53	60	65	66	48
glycitein 7-β-glucoside	39	9	11	12	13	13	9
genistin	197	88	118	131	137	144	130
daidzein	tr	7	7	10	11	7	6
glycitein	tr	tr	1	1	tr	tr	tr
genistein	1	2	2	2	2	1	2

[a] Corrected for an assumed 20% oil content. [b] Solvent: sample ratio.

aglycons were determined based on the internal standard. These response factors were used to calculate the isoflavone and isoflavone glucoside composition of various commercial soybean protein products.

RESULTS AND DISCUSSION

Shown in Figure 1 is a typical elution diagram obtained upon chromatographing an 80% methanol extract of soybean flour. This particular elution pattern is for an extract of Centex 400 flour (sample G).

Table II shows results obtained when a single lot (sample A) of hexane-defatted soybean flour was extracted with hot 80% aqueous methanol for various times at different solvent ratios. Also included from Naim et al. (1974), who used GLC, are data that have been corrected to an oil-free basis by assuming 20% oil. As seen in Table II, there is an increase in the extraction of the isoflavone glucosides with time. The largest increase is in genistin, which goes from 88 mg/100 g of meal in 1 h to 144 mg/100 g of meal in 5 h, whereas changing the solvent ratio from 15:1 to 45:1 and extracting for 5 h do not increase the amount of isoflavone glucoside found. The results indicate that the slow extraction of the isoflavone glucosides is not due to limited solubility and that a 4-h extraction appears to be sufficient for extracting the isoflavones from soybean meal.

Table III gives the amounts of isoflavone glucosides and aglycons measured in several commercial defatted soybean flour products. The glucosides daidzin and genistin account for well over 50% of the total isoflavone found in soybean flours. In sample H, these two isoflavone glucosides account for 75% of the total isoflavones measured.

Table IV gives the results of analyzing five different soybean protein concentrates (products which contain a minimum of 70% protein). Concentrates L and O were prepared by aqueous leaching of defatted soybean flours (Circle and Smith, 1972), and the amount of isoflavone measured in the sample approximates the amount of isoflavone measured in soybean flours (Table III). Concentrates K, M, and N, on the other hand, are prepared by

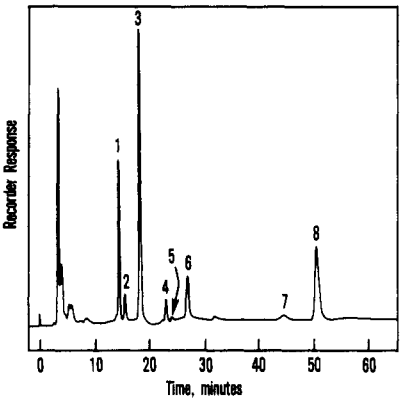

Figure 1. High-performance liquid chromatographic elution diagram of an 80% aqueous methanolic extract of texturized soybean flour. Peaks are (1) daidzin, (2) glycitein 7-β-glucoside, (3) genistin, (4) daidzein, (5) glycitein, (6) genistein, (7) coumesterol, and (8) *n*-butyrophenone.

extracting hexane-defatted soybean meals with aqueous alcohols. This alcohol treatment should remove some of the isoflavones from the meal. The results shown for samples K, M, and N in Table IV indeed show a decrease in the isoflavones.

Table V shows the results obtained when five different soybean protein isolates (products containing at least 90% protein) were analyzed for their isoflavone contents. The majority of the isoflavone measured was genistin, as was observed when soybean flours were analyzed. Although the five soybean protein isolates analyzed are manufactured by different procedures so that the end products have different characteristics, their isoflavone content is fairly constant. About 50% of the total isoflavones in hexane-defatted soybean meal is lost when soybean protein isolate is prepared. The data in Tables III and V show that isoflavone glycosides are preferentially lost in the protein isolation procedure. This decrease in the isoflavone glycosides during protein isolation may be because the glycosides are more soluble than the aglycons in water, which is used for the extraction of soybean protein.

CONCLUSIONS

A high-performance liquid chromatographic procedure has been used to quantitatively measure the isoflavone contents of commercial soybean protein products. The isoflavones in soybeans exist in several forms, i.e., as glucosides, acetyl glucosides, and aglycons. The majority of the isoflavones are present as the glycosides.

In 1980 Drane et al. (1980) showed that rations containing soybean meal prepared for rats caused the uteri of mice to increase in size and concluded soybean meal does have estrogenic activity. Earlier Bickoff et al. (1962) showed that the quantity of genistein and daidzein needed to produce a 25-mg uteri in mice was 8000 and 10 000 μg, respectively. More recent research by Kitts et al. (1980)

Table III. Isoflavone Analysis of Ten Defatted Soy Flours

	mg/100 g, as is, of flour[a]									
isoflavone	A	B	C	D	E	F	G	H	I	J
daidzin	62	61	77	61	49	55	48	77	65	62
glycitein 7-β-glucoside	18	12	22	13	12	13	11	15	6	7
genistin	127	123	146	119	102	58	98	154	142	129
daidzein	48	8	37	46	36	31	17	33	45	27
glycitein	tr	2	3	tr	3	2	tr	tr	tr	tr
genistein	40	4	21	46	27	19	21	26	38	25
total	295	210	306	285	229	178	195	305	296	250

[a] Average of two replicates. Relative standard error per mean is 9.6%. Least significant ratio (0.05 level) of two means is 1.2.

Table IV. Isoflavone Analysis of Five Soybean Protein Concentrates

isoflavone	mg/100 g, as is, of concentrate[a]				
	K	L	M	N	O
daidzin	3	59	9	4	76
glycitein 7-β-glucoside	1	22	2	1	13
genistin	4	124	19	6	191
daidzein	11	20	12	2	11
glycitein	1	tr	tr	1	4
genistein	1	22	1	2	22
total	21	247	43	16	317

[a] Average of two replicates. Relative standard error per mean is 13.7%. Least significant ratio (0.05 level) of two means is 1.5.

Table V. Isoflavone Analysis of Five Soybean Protein Isolates

isoflavone	mg/100 g, as is, of isolate[a]				
	P	Q	R	S	T
daidzin	16	14	23	20	30
glycitein 7-β-glucoside	3	4	4	3	6
genistin	67	59	80	66	55
daidzein	8	12	18	10	21
glycitein	2	1	2	1	3
genistein	22	13	18	5	17
total	118	103	145	105	132

[a] Average of two replicates. Relative standard error per mean is 18.1%. Least significant ratio (0.05 level) of two means is 1.6.

indicates that lesser amounts of genistein may be needed to cause an effect in rat uteri. Our studies show low levels of daidzein and genistein in soybean protein products but large amounts of the isoflavone glucosides. The total isoflavone glucosides and aglycons measured in this study in hexane-defatted soybean meal appears to be approximately 2500 μg/g. Further research is needed to study these soybean constituents as a source of estrogenic response in animals.

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Effect of pH on the Extraction and Fractionation of Dry Matter and Crude Protein from Coastal Bermuda Grass and White Clover

John J. Evans

Freeze-dried Coastal Bermuda grass (CBG) and white clover (WC) were extracted at pHs ranging from 4 to 10 and fractionated into four distinct fractions: chloroplastic (CHL), cytoplasmic (CYT), nonprotein nitrogen (NPN), and residue (RES). Dry matter (DM) and crude protein (CP) distributions in the fractions were influenced by pH. At pH 4, the greatest amount of CHL protein was extracted from CBG while the least amount was extracted from WC. At pHs ranging from 6 to 10, the CHL CP extracted remained constant for each forage with WC having twice the CHL CP as CBG. CYT CP extractability exhibited a quadratic effect ($P < 0.001$) due to pH; the pH optima for extraction of CYT proteins occurred at pHs 7 and 8 for CBG and WC, respectively. The amounts of CYT CP extracted from CBG and WC at their optimal pH were equivalent. The NPN fractions increased in CP with increasing pH while the CP in the RES fractions decreased with increasing pH for both forages. In general, the DM distribution paralleled the CP distribution.

The economical production of leaf protein concentrates from forages is desirable since forages can yield more dry matter and crude protein than any other crop (Pirie, 1979). The fractionation of forage proteins into green chloroplastic fractions for use in animal feeds and nearly white cytoplasmic fractions for human use has increased the importance of forages as sources of protein (Subba Rau et al., 1969; Evans et al., 1974; Horigome, 1977; Hanna and Ogden, 1980). Consequently, many different extraction and fractionation procedures have been described (Spencer et al., 1970, 1971; Pirie, 1971; Fishman and Burdick, 1977; Ostrowski, 1979) in an attempt to efficiently extract proteins from different plants. Chloroplastic and cytoplasmic proteins have been separated mainly on the basis of differential heat treatment of the expressed plant juices (Byers, 1967; Lexander et al., 1970; de Fremery et al., 1973; Edwards et al., 1975; Miller et al., 1975). These extraction

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